

PCT

WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau



AG

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6: C12Q 1/68, C07H 21/00	A2	(11) International Publication Number: WO 96/19587
		(43) International Publication Date: 27 June 1996 (27.06.96)
(21) International Application Number: PCT/US95/16627		(81) Designated States: CA, JP, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).
(22) International Filing Date: 20 December 1995 (20.12.95)		
(30) Priority Data: 08/362,281 22 December 1994 (22.12.94) US		Published <i>Without international search report and to be republished upon receipt of that report.</i>
(71) Applicant: ABBOTT LABORATORIES [US/US]; CHAD 0377/AP6D-2, 100 Abbott Park Road, Abbott Park, IL 60064-3500 (US).		
(72) Inventors: JOU, Tsung-Hui, K.; 917 Springhaven Drive, Libertyville, IL 60048 (US). HOIJER, Joaneil, V.; 111 West Lillian Avenue, Arlington Heights, IL 60004 (US).		
(74) Agents: YASGER, Paul, D. et al.; Abbott Laboratories, CHAD 0377/AP6D-2, 100 Abbott Park Road, Abbott Park, IL 60064-3500 (US).		

(54) Title: METHODS OF IMMOBILIZING OLIGONUCLEOTIDES TO SOLID SUPPORT MATERIALS AND METHODS OF USING SUPPORT BOUND OLIGONUCLEOTIDES

(57) Abstract

The present invention provides a method for directly immobilizing an oligonucleotide to a support material. The method comprises the steps of contacting a solution of oligonucleotides with a solid support material and drying the oligonucleotide solution to the support material. Oligonucleotides and the solid support materials to which they are immobilized can be employed as capture reagents for immobilizing nucleic acid sequences which are complementary to the immobilized oligonucleotides. Hence, the hybridization capacity of directly immobilized oligonucleotides is maintained. Also provided are methods for determining the presence or amount of nucleic acid sequences in a test sample.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AT	Austria	GB	United Kingdom	MR	Mauritania
AU	Australia	GE	Georgia	MW	Malawi
BB	Barbados	GN	Guinea	NE	Niger
BE	Belgium	GR	Greece	NL	Netherlands
BF	Burkina Faso	HU	Hungary	NO	Norway
BG	Bulgaria	IE	Ireland	NZ	New Zealand
BJ	Benin	IT	Italy	PL	Poland
BR	Brazil	JP	Japan	PT	Portugal
BV	Bolivia	KE	Kenya	RO	Romania
CA	Canada	KG	Kyrgyzstan	RU	Russian Federation
CF	Central African Republic	KP	Democratic People's Republic of Korea	SD	Sudan
CG	Congo	KR	Republic of Korea	SE	Sweden
CH	Switzerland	KZ	Kazakhstan	SI	Slovenia
CI	Côte d'Ivoire	LJ	Liechtenstein	SK	Slovakia
CM	Cameroon	LK	Sri Lanka	SN	Senegal
CN	China	LU	Luxembourg	TD	Chad
CS	Czechoslovakia	LV	Larvia	TG	Togo
CZ	Czech Republic	MC	Monaco	TJ	Tajikistan
DE	Germany	MD	Republic of Moldova	TT	Trinidad and Tobago
DK	Denmark	MG	Madagascar	UA	Ukraine
ES	Spain	ML	Mali	US	United States of America
FI	Finland	MN	Mongolia	UZ	Uzbekistan
FR	France			VN	Viet Nam
GA	Gabon				

METHODS OF IMMOBILIZING OLIGONUCLEOTIDES TO SOLID SUPPORT MATERIALS AND METHODS OF USING SUPPORT BOUND OLIGONUCLEOTIDES

5 This is a continuation-in-part application of co-pending U.S. Patent Application Serial No. 08/311,462 filed on September 22, 1994.

Field of the Invention

10 The present invention relates to oligonucleotides. In particular, the invention relates to the immobilization of short oligonucleotides to support materials.

Background of the Invention

15 Amplification reactions such as the ligase chain reaction (LCR) which is described in European Patent Applications EP-A-320-308, the gap ligase chain reaction (GLCR) which is described in EP-A-439-182, and the polymerase chain reaction (PCR) which is described in U.S. Patents Numbered 4,683,202 and 4,683,195 are well known in the art. Such nucleic acid amplification processes are becoming useful clinical diagnostic tools to, for example, 20 construct assays which detect infectious organisms in a test sample.

Amplification reactions have also found utility in research and development fields as well as forensic fields.

Nucleic acid amplification techniques typically generate copies of a target nucleic acid sequence and the presence or amount of the target sequence copies 25 can be detected using immunological assay techniques. For example, target sequence copies can be contacted with a "capture reagent" which comprises a substantially solid support material such as, for example, a suspension of microparticles coated with an oligonucleotide (variably referred to as a capture oligonucleotide) which specifically hybridizes with the target sequence copies. 30 In this manner, target or products of an amplification reaction can be immobilized to a capture reagent by virtue of a target sequence copy's hybridization with the capture oligonucleotide. Once the target sequences are immobilized to the capture reagent, they can easily be separated from, for example, extraneous reactants, by separating the solid support from the reaction mixture such as by washing or filtration. The presence or amount of the 35 amplified sequences which may be immobilized to the capture reagent can be

-2-

detected by contacting the captured target sequence with a "conjugate". A conjugate can comprise a detectable moiety which is attached to specific binding pair member that also specifically binds the amplified target sequences which are immobilized to the capture reagent. By detecting the presence of the detectable 5 moiety, the presence or amount of the target sequences can be determined.

One known method of immobilizing an oligonucleotide to a support material uses chemical crosslinking agents. Typically, crosslinking agents covalently bind a support material and an oligonucleotide to form a linking arm which attaches the oligonucleotide to the support material. For example, U.S. 10 Patent No. 4,948,882 discloses compounds which can be employed to covalently link an oligonucleotide to a solid support material. However, chemically crosslinking an oligonucleotide to a support material generally is a time consuming process which requires modifications to the base pairs comprising the oligonucleotide.

Another method of immobilizing an oligonucleotide to a support material which is described in Saiki, R.K., *et al.*, Proc. Natl. Acad. Sci. USA, 86:6230-6234 (1989) involves the use of "tails". Tails are extensions of oligonucleotides that are typically around fifteen base pairs or more in length. An oligonucleotide's tail preferentially binds solid support material and, similarly to 20 a crosslinking agent, leaves the oligonucleotide free of the support material and available for hybridization. Unfortunately, tails, which are themselves nucleic acids, sometimes interfere with the oligonucleotide's ability to specifically hybridize to a nucleic acid sequence.

As evidenced by the aforementioned methods of immobilizing 25 oligonucleotides to support materials, it has been accepted that relatively short oligonucleotides having between about 5 and about 50 base pairs cannot be attached directly to a solid support material without impairing the hybridization capacity of the oligonucleotide. Accordingly, known methods of attaching oligonucleotides to support materials indirectly bind oligonucleotides to support 30 materials. By indirect attachment, the oligonucleotide itself is not bound to the support material and, theoretically, is free to hybridize to another nucleic acid sequence.

-3-

Summary of the Invention

The present application describes a method for directly and non-covalently immobilizing an oligonucleotide to a support material. According to the instant method, immobilization of an oligonucleotide to a support material is effected quickly and without chemical modifications to the bases comprising the oligonucleotide. Importantly, the hybridization capacity of a directly immobilized oligonucleotide is not impaired. Oligonucleotides and support materials to which they are immobilized can be employed as capture reagents for immobilizing nucleic acid sequences which are complementary to the oligonucleotides bound to the support material.

The method comprises the steps of contacting a solution of oligonucleotides with a solid support material and drying the oligonucleotide solution upon the support material. The oligonucleotides in solution can be in the range of between about 5 nucleotides and about 30 nucleotides in length. Additionally, it has been discovered that the affinity of an oligonucleotide for a support material can be enhanced by modifying the oligonucleotides. The method may further comprise a baking step and/or an overcoating step.

The presence or amount of the support bound oligonucleotides can be detected by contacting the solid support material, and the oligonucleotides immobilized thereon, with a conjugate and detecting a measureable signal as an indication of the presence or amount of the immobilized oligonucleotides.

According to another embodiment, the solid support material, and the immobilized oligonucleotides thereon, can be contacted with a conjugate and a measurable signal can be detected as an indication of the presence or amount of the conjugate.

According to yet another embodiment, support bound oligonucleotides can be contacted with a test sample suspected of containing nucleic acid sequences which are complementary to the immobilized oligonucleotides to form hybridization complexes. The hybridization complexes can then be contacted with a conjugate and a measureable signal can be detected as an indication of the presence or amount of any complementary nucleic acid sequences in the test sample.

-4-

Brief Description of the Drawings

Figure 1 illustrates the principles of total internal reflectance (TIR).

Figure 2A, 2B and 2C are, respectively, perspective, side and cross section views of a waveguide device. Figure 2C is an enlarged cross section taken along line C-C of Figure 2B.

Figures 3, 4A-4D, 5A-5B, 6A-6C, and 7A-7B are printed representations of results obtained for assays using a capture reagent comprising oligonucleotides which were immobilized to a support material as taught herein. Details of these printed data are found in Example 1 through Example 4.

10

Detailed Description of the Invention

Despite previous teachings, it was surprisingly discovered that an oligonucleotide comprising between about 5 nucleotides and about 50 nucleotides can be directly and non-covalently attached to a solid support material without impairing the hybridization capacity of the immobilized oligonucleotide. While the mechanism by which oligonucleotides directly adhere to a solid surface is not completely understood, directly attaching an oligonucleotide to a solid surface means that an oligonucleotide becomes immobilized to a support material in a manner similar to adsorption. Further, direct attachment of an oligonucleotide to a solid surface does not require crosslinking agents, tails or additional nucleic acid sequences to affect the immobilization. Importantly, when oligonucleotides are directly attached to a solid surface as taught herein, the oligonucleotides can specifically pair or hybridize with a complementary nucleic acid sequence.

25

Oligonucleotides which are immobilized as taught herein can be employed to capture or otherwise immobilize complementary nucleic acid sequences such as, for example, 2'-deoxyribonucleic acid (DNA), ribonucleic acid (RNA), peptide nucleic acid (PNA - as described WO 93/25706) and the like to support materials to which the oligonucleotides are immobilized. Once complementary sequences hybridize to the immobilized oligonucleotides, their presence can be detected using methodologies well known in the art.

30

For example, a capture reagent comprising a solid support having oligonucleotides directly immobilized thereon can be contacted with a test sample. The test sample can be any liquid suspected of containing a nucleic acid sequence which can specifically hybridize with the immobilized oligonucleotides. The capture reagent and test sample can be contacted for a time

35

-5-

and under conditions suitable for allowing nucleic acids in the test sample, if any, and the oligonucleotides to hybridize and thereby form hybridization complexes. The hybridization complexes, if any, can be contacted with a conjugate for a time and under conditions sufficient to enable the conjugate to specifically bind any hybridization complexes. A signal can then be detected as an indication of the presence or amount of any nucleic acid sequences which may have been present in the test sample.

Immobilized oligonucleotides as taught herein can also be employed in a "one-step" assay configuration. According to such a configuration, a test sample

suspected of containing nucleic acids which are complementary to the immobilized oligonucleotides can be contacted with a conjugate for a time and under conditions suitable for allowing the conjugate to bind any nucleic acid sequences which may be present in the test sample to form conjugate/nucleic acid complexes. Alternatively, the nucleic acids which may be present in a test sample may comprise a detectable moiety. Nucleic acid sequences can be labeled or conjugated with a detectable moiety through, for example, nick translation whereby labeled nucleotides are incorporated into a target sequence.

Conjugate/nucleic acid complexes or nucleic acids which comprise a detectable moiety can then be contacted with the support bound oligonucleotides to form conjugate/nucleic acid/oligonucleotide complexes or nucleic acid/oligonucleotide complexes. A signal can then be detected as an indication of the presence or amount of any nucleic acid sequences present in the test sample.

In a preferred embodiment, a method for quickly detecting the presence of an oligonucleotide in a test sample is provided. According to this embodiment, a sample which is suspected of containing oligonucleotides can be contacted with a support material and the oligonucleotides which may be present in the test sample can be immobilized to the support material as taught herein. A conjugate can then be contacted with the immobilized oligonucleotides for a time and under conditions for allowing the conjugate to bind the immobilized oligonucleotides. A signal can then be detected as an indication of the presence or amount of any oligonucleotides which may have been present in the test sample.

The period for which oligonucleotides which are immobilized as taught herein are contacted with, for example, a test sample, conjugate/nucleic acid complexes, or a conjugate is not important. However, it is preferred that such a

-6-

contact period be kept to a minimum for example less than 30 minutes, more preferably less than 15 minutes and most preferably less than 10 minutes.

Those skilled in the art will understand that a conjugate may comprise a detectable moiety attached to specific binding pair member. Detectable moieties 5 may include any compound or conventional detectable chemical group having a detectable and measurable physical or chemical property variably referred to as a signal. Such detectable groups can be, but are not intended to be limited to, enzymatically active groups such as enzymes and enzyme substrates, prosthetic groups or coenzymes; spin labels; fluorescent molecules such as fluorescers and 10 fluorogens; chromophores and chromogens; luminescent molecules such as luminescers, chemiluminescers and bioluminescers; phosphorescent molecules; specifically bindable ligands such as biotin and avidin; electroactive species; radioisotopes; toxins; drugs; haptens; polysaccharides; polypeptides; liposomes; colored or fluorescent particles; colored or fluorescent microparticles; colloidal 15 particles such as selenium colloid or gold colloid; and the like. Additionally, a detectable moiety can comprise, for example, a plurality of fluorophores immobilized to a polymer such as that described in co-owned and co-pending U.S. Patent Application Serial No. 091,149 filed on July 13, 1993, which is herein incorporated by reference. The detectable physical or chemical property 20 associated with a detectable moiety can be detected visually or by an external means. Specific binding member is a well known term and generally means a member of a binding pair, i.e., two different molecules where one of the molecules through chemical or physical means specifically binds to the other molecule. In addition to antigen and antibody specific binding pairs, other 25 specific binding pairs include, but are not intended to be limited to, avidin and biotin, antibody and hapten, complementary nucleotide sequences or complementary nucleic acid sequences such as DNA, RNA or PNA, an enzyme cofactor or substrate and an enzyme, a peptide sequence and an antibody specific for the sequence or an entire protein, dyes and protein binders, peptides and 30 specific protein binders (e. g., ribonuclease, S-peptide and ribonuclease S-protein), and the like. Furthermore, binding pairs can include members that are analogs of the original binding member, for example, an analyte-analog or a binding member made by recombinant techniques or molecular engineering. Thus, PNAs are specific binding members for DNA or RNA. If the binding 35 member is an immunoreactant it can be, for example, a monoclonal or polyclonal antibody, a recombinant protein or recombinant antibody, a chimeric antibody, a

-7-

mixture(s) or fragment(s) of the foregoing. Detectable moieties can be attached to specific binding pair members through any chemical means and/or physical means that do not destroy the specific binding properties of the specific binding member or the detectable properties of the detectable moiety.

- 5 Preferably the method herein provided is employed to immobilize oligonucleotides to a glass surface which is then employed in a waveguide configuration such as that taught in co-owned and co-pending U.S. Patent Application Serial No. 08/311,462 filed on September 22, 1994 and entitled "Light Scattering Optical Waveguide Method for Detecting Specific Binding
- 10 Events" which is herein incorporated by reference. A waveguide device's ability to be employed in an immunoassay or hybridization type format is based upon a phenomenon called total internal reflection (TIR) which is known in the art and is described with reference to Figure 1. TIR operates upon the principle that light 10 traveling in a denser medium 12 (i.e. having the higher refractive index, N₁) and striking the interface 14 between the denser medium and a rarer medium 16 (i.e. having the lower refractive index, N₂) is totally reflected within the denser medium 12 if it strikes the interface at an angle, θ_R, greater than the critical angle, θ_C where the critical angle is defined by the equation:

$$\theta_C = \arcsin (N_2/N_1)$$

- 20 Under these conditions, an electromagnetic waveform known as an "evanescent wave" is generated. As shown in Figure 1, the electric field associated with the light in the denser medium forms a standing sinusoidal wave 18 normal to the interface. The evanescent wave penetrates into the rarer medium 16, but its energy E dissipates exponentially as a function of distance Z from the interface
- 25 as shown at 20. A parameter known as "penetration depth" (d_p- shown in Figure 1 at 22) is defined as the distance from the interface at which the evanescent wave energy has fallen to 0.368 times the energy value at the interface. [See, Sutherland et al., *J. Immunol. Meth.*, 74:253-265 (1984) defining d_p as the depth where E = (e⁻¹)·E₀. Penetration depth is calculated as
- 30 follows:

$$d_p = \frac{\lambda/N_1}{2\pi\{\sin^2\theta_R - (N_2/N_1)^2\}^{1/2}}$$

- Factors that tend to increase the penetration depth are: increasing angle of incidence, θ_R; closely matching indices of refraction of the two media (i.e. N₂/N₁ → 1); and increasing wavelength, λ. For example, if a quartz TIR element (N₁ = 1.46) is placed in an aqueous medium (N₂ = 1.34), the critical

-8-

angle, θ_C , is 66° ($= \arcsin 0.9178$). If 500 nm light impacts the interface at $\theta_R = 70^\circ$ (i.e. greater than the critical angle) the d_p is approximately 270 nm.

TIR has also been used in conjunction with light scattering detection in a technique referred to as Scattered Total Internal Reflectance ("STIR"). See, 5 e.g., U.S. Patents 4,979,821 and 5,017,009 to Schutt, et al and WO 94/00763 (Akzo N. V.). According to this technique, a beam of light is scanned across the surface of a TIR element at a suitable angle and the light energy is totally reflected except for the evanescent wave. Particles such as red blood cells, 10 colloidal gold or latex specifically bound within the penetration depth will scatter the light and the scattered light is detected by a photodetection means.

Figures 2A-2C illustrate a waveguide device 30 comprising a planar waveguide element 32 and a parallel planar plate 34. The waveguide element thus has parallel surfaces 36 and 38 as well as a light-receiving edge 40. Similarly, the plate 34 has parallel surfaces 42 and 44. The waveguide element 15 32 and the plate 34 are held together in spaced parallel fashion, such that the element surfaces 38 and the plate surface 42 define a narrow channel 46. The element and plate may be held together by any convenient means, including adhesive means 48 consisting of double stick tape disposed along the edges of the element and plate. The channel 46 is preferably rather small so as to enable 20 capillary transfer of a fluid sample therethrough. For example, the height should be less than about 1mm, preferably less than about 0.1mm.

The element 32 should be made of an optically transparent material such as glass, quartz, plastics such as polycarbonate, acrylic, or polystyrene. The refractive index of the waveguide must be greater than the refractive index of the 25 sample fluid, as is known in the art for effecting total internal reflectance. For an aqueous sample solution, the refractive index, n, is about 1.33, so the waveguide typically has a refractive index of greater than 1.35, usually about 1.5 or more. The waveguide may be a piece of plastic or glass, for example, a standard glass microscope slide or cover slip may be used.

30 The plate 34 may be constructed of similar materials. As seen in Figures 2A and 2B, the light receiving end 40 of the waveguide element 32 is disposed in a narrow slit 50 of a mask 52 in order to minimize the effects of stray light originating from the light source 54. Minimization of stray light is also improved by the use of light absorbing materials.

35 Light source 54 for generating the incident light beam may be a source of electromagnetic energy, including energy in the visible, ultra-violet, and near-IR

-9-

spectra. The term "light" is thus construed quite broadly and is not confined to the visible range, except in cases where detection is made visually. Non-visible wavelengths are detected by detectors optimized for the particular wavelength as is well known in the art. The light may be monochromatic or polychromatic, 5 collimated or uncollimated, polarized or unpolarized. Preferred light sources include lasers, light emitting diodes, flash lamps, arc lamps, incandescent lamps and fluorescent discharge lamps. The light source used to illuminate the waveguide element can be a low wattage helium-neon laser. For a portable disposable such as that described in example 1 below, the light source can be a 10 small incandescent light bulb powered by a battery, such as is used in pocket flashlight. Preferably, the light source includes potentiometer means for varying the intensity of the light source. Alternatively, filters and/or lenses may be employed to adjust the intensity to a suitable level.

Detection means may be employed to determine light scattering produced 15 by a light scattering label (LSL). As seen best in Figure 2A, a LSL may be immobilized to surface 38 of waveguide element 32 via interactions between specific binding members such as, for example, that between an immobilized oligonucleotide and a cognate DNA sequence. A LSL is a molecule or a material, often a particle, which causes incident light to be scattered elastically, 20 i.e. substantially without absorbing the light energy. Exemplary LSLs include colloidal metal and non-metal labels such as colloidal gold or selenium; red blood cells; and dyed plastic particles made of latex, polystyrene, polymethylacrylate, polycarbonate or similar materials. The size of such particulate labels ranges from 10 nm to 10 μm , typically from 50 to 500 nm, and preferably from 70 to 25 200 nm. The larger the particle, the greater the light scattering effect, but this is true of both bound and bulk solution particles, so background also increases with particle size. Suitable particle LSLs are available from Bangs Laboratories, Inc., Carmel, IN, USA.

Instrumentation and visual detection means may be employed to 30 determine the degree of light scattering produced by a LSL. Light scattering events across the entire waveguide can be monitored essentially simultaneously, whether by the eye and brain of an observer or by photodetection devices including CCD cameras which form images that are digitized and processed using computers.

35 As previously mentioned, immobilizing oligonucleotides to support materials according to the instant invention comprises contacting a support

-10-

material with an oligonucleotide solution and drying the solution upon the support material.

Support materials or solid supports to which oligonucleotides can be immobilized are well known in the art and include materials that are substantially insoluble. Porous materials can serve as solid supports and may include, for example, paper; nylon; and cellulose as well as its derivatives such as nitrocellulose. Smooth polymeric and nonpolymeric materials are also suitable support materials and include, but are not intended to be limited to, plastics and derivatized plastics such as, for example, polycarbonate, polystyrene, and polypropylene; magnetic or non-magnetic metal; quartz and glass. Preferably, quartz, glass or nitrocellulose is employed as a support material. Solid supports can be used in many configurations well known to those skilled in the art including, but not limited to, test tubes, microtiter wells, sheets, films, strips, beads, microparticles, chips, slides, cover slips, and the like.

Oligonucleotides according to the invention will be understood to mean a sequence of DNA, RNA or PNA. The length of an oligonucleotide which is immobilized to a support material is largely a matter of choice for one skilled in the art and is typically based upon the length of a complementary sequence of, for example, DNA, RNA, or PNA which will be captured. While the length of an immobilized oligonucleotide is typically between about 5 and about 50 bases, preferably, the length of an immobilized oligonucleotide is between about 5 and about 30 bases, more typically between about 10 and about 25 bases.

Synthesis of oligonucleotides is fairly routine using automated synthesizers. If desired, automated synthesizers can produce oligonucleotides which are modified with terminal amines or other groups. A useful review of coupling chemistries is found in Goodchild, Bioconjugate Chemistry, 1(3):165-187 (1990).

Modified oligonucleotides may have a greater affinity for solid supports than unmodified oligonucleotides. Methodologies for modifying an oligonucleotide are well known and may include the addition of chemical groups such as amines, or haptens such as fluorescein to an oligonucleotide. Such modifications do not provide for covalent linkages between a support material and an oligonucleotide, but nevertheless have been found to increase the affinity of oligonucleotides for support materials. Modifications are particularly effective when made to the 3' or 5' ends of an oligonucleotide.

-11-

The oligonucleotide solution that is contacted with a solid support may comprise oligonucleotides which are in solution. The concentration of the oligonucleotides in the solution is largely a matter of choice for one skilled in the art and is typically based upon how the immobilized oligonucleotides will be employed. Generally the oligonucleotide solution will have an oligonucleotide concentration of between about 1 μ M and about 1 mM, preferably between about 5
5 20 μ M and about 250 μ M. However, as previously mentioned, modified oligonucleotides have been found to have a greater affinity for support materials than unmodified oligonucleotides. Accordingly, modified oligonucleotides can 10 be employed in lower concentration ranges than unmodified oligonucleotides.

The pH of the oligonucleotide solution may be between about 6.5 and about 8.0, preferably between about 7.0 and about 7.5. Additionally, oligonucleotide solutions are preferably saline and the sodium chloride concentration of such a solutions can vary greatly but is typically between about 15 75 mM and about 2 M, preferably between about 100 mM and about 1 M, and most preferably between about 120 mM and about 500 mM.

Buffering systems may optionally be included in the oligonucleotide solution. Buffering system are well known and generally comprise aqueous solutions of compounds which resist changes in a solution's hydrogen ion 20 concentration. Examples of buffering systems include, but are not intended to be limited to, solutions of a weak acid or base and salts thereof such as, for example, acetates, borates, phosphates, phthalates, citrates, carbonates and the like. Preferably, the buffering system comprises between about 5 mM and about 250 mM tris®, sodium citrate, or sodium phosphate, more preferably between about 10 mM and about 200 mM tris®, sodium citrate, or sodium phosphate and most preferably between about 10 mM and about 175 mM tris®, sodium citrate, 25 or sodium phosphate.

The amount of oligonucleotide solution which is applied or "spotted" upon a solid support need be large enough only to capture sufficient 30 complementary sequences to enable detection of, for example, a captured sequence or conjugate. This is dependent in part on the density of support material to which the capture oligonucleotide is immobilized. For example, areas of as little as 150 μ m in diameter may be employed. Such small areas are preferred when many sites on a support material are spotted with oligonucleotide 35 solution(s). The practical lower limit of size is about 1 μ m in diameter. For visual detection, areas large enough to be detected without magnification are

-12-

desired; for example at least about 1 to about 50 mm², up to as large as 1 cm² or even larger. There is no upper size limit except as dictated by manufacturing costs and user convenience.

Once an oligonucleotide solution is contacted with a solid support, 5 evaporation is the preferred drying method and may be performed at room temperature (about 25°C). When desired, the evaporation may be performed at an elevated temperature, so long as the temperature does not significantly inhibit the ability of the oligonucleotides to specifically hybridize with complementary sequences.

10 The process of immobilizing oligonucleotides to a solid support may further comprise "baking" the support material and the oligonucleotide solution thereon. Baking may include subjecting the solid phase and oligonucleotide solution residue, to temperatures between about 60°C and about 95°C, preferably between about 70°C and about 80°C. The baking time is not critical 15 and preferably lasts for between about 15 minutes and about 90 minutes. Baking is particularly preferred when porous support materials such as, for example, nitrocellulose are employed.

An overcoating step may optionally be employed in the method herein provided when porous support materials are employed, but when smooth 20 polymeric or nonpolymeric supports are employed in a waveguide format, an overcoating step is particularly preferred. Overcoating typically comprises treating the support material so as to block non-specific interactions between the support material and complementary sequences which may be in a fluid sample. It is preferred that the overcoating or blocking material is applied before the 25 oligonucleotide solution has been dried upon the support material. Suitable blocking materials are casein, zein, bovine serum albumin (BSA), 0.5% sodiumdodecyl sulfate (SDS), and 1X to 5X Denhardt's solution (1X Denhardt's is 0.02% Ficoll, 0.02% polyvinylpyrrolidone and 0.2 mg/ml BSA). Other blockers can include detergents and long-chain water soluble polymers.

30 Casein has been found to be a preferred blocking material and is available from Sigma Chemical, St Louis, MO. Casein belongs to a class of proteins known as "meta-soluble" proteins (see, e.g., U.S. Patent 5,120,643 to Ching, et al, incorporated herein by reference) which are preferably treated to render them more soluble. Such treatments include acid or alkaline treatment and are believed to perform cleavage and/or partial hydrolysis of the intact protein. 35 Casein is a milk protein having a molecular weight of about 23,600 (bovine beta-

-13-

casein), but as used herein, "casein" or "alkaline treated" casein both refer to a partially hydrolyzed mixture that results from alkaline treatment as described in example 1 of US Patent 5,120,643. An electrophoresis gel (20% polyacrylamide TBE) of the so-treated casein shows a mixture of fragments

- 5 predominantly having molecular weight less than 15,000, as shown by a diffused band below this marker.

EXAMPLES

10 **Example 1. DNA Hybridization Assay**

A. DNA Waveguide Construction

DNA waveguides for the detection of human genetic mutations that cause cystic fibrosis were constructed from glass substrates 1 cm square.

- 15 Oligonucleotides were immobilized to the glass to provide multiple capture sites in the reactive surface. In particular, nine different oligonucleotides, designated CAT01 through CAT09 (SEQ ID Nos. 1 - 9) were applied to the glass surface of the waveguide to form a 3 x 3 array pattern such that the CAT# corresponded to the position occupied by the same number on a standard touch-tone telephone. DNA spots were about 2 mm in diameter and about 2 mm apart. The sequence 20 and mutation site of CAT01 through CAT09 (SEQ ID Nos. 1 - 9) are shown in Table 1.1.

TABLE 1.1

SEQ ID No.	Oligo Designation	Sequence 5'-----to-----3'	Mutation Designation
1	CAT01	TATCATCTTGGTGT-NH ₂	ΔF508WT
2	CAT02	AAATATCATTGGTGT-T-NH ₂	ΔF508
3	CAT03	AGTGGAGGTCAACGA-NH ₂	G551D WT
4	CAT04	AGTGGAGATCAACGA-NH ₂	G551D
5	CAT05	AGGTCAACGAGCAAG-NH ₂	R553X WT
6	CAT06	AGGTCAATGAGCAAG-NH ₂	R553X
7	CAT07	TGGAGATCAATGAGC-NH ₂	G551D + R553X
8	CAT08	TGGAGATCAACGAGC-NH ₂	G551D + R553X WT
9	CAT09	TGGAGGTCAATGAGC-NH ₂	G551D WT+ R553X

-14-

The human genetic mutations are indicated by standard notation. For example, $\Delta F508$ indicates a 3 base pair deletion at position 508 of the cystic fibrosis transmembrane conductance regulator polypeptide (J. Zielenski, et al. Genomics 10:214-228, 1991). The "WT" indicates the wild type or normal sequence at this position. The DNA solutions were prepared by Synthecell (Columbia, MD) and were diluted 1:20 into PBS (phosphate buffered saline, pH 7.4) buffer and applied to the glass surface of the waveguide using the blunt end of a drill bit approximately 1 mm in diameter. DNA was immobilized on a clean glass surface or to a glass surface previously coated with 0.05% casein; hybridization results were indistinguishable. The final concentrations of DNA applied to the glass surface of the waveguide ranged from a high value of 14 μM for CAT02 to a low of 0.9 μM for CAT08 and was determined by comparison to the concentration of starting material received from Synthecell. After application, the DNA solutions were allowed to dry on the chip at room temperature or, on humid days between about 35% and 80% relative humidity, in an incubator set at 50-70 °C until dry (about 10 minutes). This procedure formed nine "spots" or hybridization capture sites in the 3 x 3 array described above. Another glass cover slip created a channel to hold the conjugate solution. The two cover slips were offset and held together by double-sided tape (Arcare 7710B, Adhesives Research Inc., Glen Rock, Penn) so as to form a channel approximately 16 mm wide and approximately 75 μm thick (the thickness of the double sided tape). The channel held approximately 25 μl in volume.

B. Hybridization

To evaluate DNA waveguide performance, nine additional oligonucleotides, CAT21B through CAT29B (SEQ ID Nos. 10-18) were synthesized by Synthecell with a biotin label on the 3' end. The sequences of the test DNA oligonucleotides are listed in Table 1.2.

-15-

TABLE 1.2

SEQ ID No.	Oligonucleotide Designation	Sequence 5'-----3'
10	CAT21B	ACACCAAAGATGATA-biotin
11	CAT22B	AACACCAATGATATT-biotin
12	CAT23B	TCCGTTGACCTCCACT-biotin
13	CAT24B	TCCGTTGATCTCCACT-biotin
14	CAT25B	CTTGCTCGTTGACCT-biotin
15	CAT26B	CTTGCTCATTGACCT-biotin
16	CAT27B	GCTCATTGATCTCCA-biotin
17	CAT28B	GCTCGTTGATCTCCA-biotin
18	CAT29B	GCTCATTGACCTCCA-biotin

The oligonucleotides were designed and named such that CAT21B (SEQ ID No. 10) is complementary to CAT01 (SEQ ID No. 1), CAT22B (SEQ ID No. 11) is complementary to CAT02 (SEQ ID No. 2), et cetera to CAT29 (SEQ ID No. 18) which is complementary to CAT09 (SEQ ID No. 9). The concentrations varied from a high of 473 µM for CAT25B (SEQ ID No. 14) to a low of 151 µM for CAT27B (SEQ ID No. 16). Each of the nine DNA samples were diluted 1 µl into 1 ml of hybridization buffer (1% casein, 10 mM Tris pH 7.4, 15 mM NaCl), and a different one was applied to each of the nine different DNA waveguides and incubated at room temperature (approximately 23 °C) for 5 minutes. The surface of the DNA waveguides were washed with PBS using a wash bottle and then stored under PBS until detection of hybridization.

15 C. Detection of Hybridization

The waveguide was illuminated with a light source comprising a 150 watt incandescent bulb with a ca. 2 mm slit aperture. The waveguide was inserted into the light source slit so that light was shone into the 2 mm thick light receiving edge of the waveguide (see figure 2A). The waveguide was inserted into the slit at approximately 45° relative to the mask.

Hybridization of the nine different biotin labeled DNA's was detected in the waveguide by light that was scattered from a selenium anti-biotin conjugate. Colloidal selenium particle as described in US Patent No. 4,954,452 to Yost, et al. having a 32 O.D. concentration, at the absorption maximum wavelength of 546 nm, was used to manufacture the conjugate. The selenium conjugate was

-16-

- prepared by addition of 2.5 µl of anti-biotin antibody (polyclonal rabbit anti-biotin antibody, 1.13 mg/ml in PBS, pH 7.4- see EP 0 160 900 B1 to Mushahwar, et al., corresponding to US Serial No. 08/196,885 which is herein incorporated by reference) to 1 ml of the selenium colloid, followed by addition
5 of 30 µl of bovine serum albumin (powder BSA dissolved in water to give a 20% w/v solution). Fifty µl of the conjugate solution was applied to the surface of the DNA waveguide and light was directed into the side of the waveguide to observe binding of selenium to the various DNA capture sites. Positive hybridization was visible at many sites within 1 minute. The DNA waveguides were washed with
10 PBS to remove excess selenium conjugate, illuminated to effect waveguide excited light scattering, and imaged. This visual signal was recorded using a standard 8 bit CCD (charged coupled device) camera (Cohu model 4815 Cohu, Inc., San Diego, CA). A digital representation of the image was created using a frame grabber (Imaging Technology Incorporated, PC VISION plus Frame
15 Grabber Board; Woburn, Mass) in a Compaq DeskPro 386/20c (Compaq Computer Corporation, Houston, TX). The digitized image data file was converted and imported into Publishers PaintBrush software (ZSoft Corp., Atlanta, Georgia) from which the image was printed on a 300 dpi resolution printer. The printed image is shown as Figure 3.
- 20 The entire pattern of DNA hybridization was detected using the waveguide in a single image measurement and allowed determination of the DNA sequence of the oligo applied to the waveguide. In the case of CAT21B (SEQ ID No. 10) and CAT22B (SEQ ID No. 11) (first two frames of Figure 4), the hybridization pattern was relatively simple because there was negligible
25 sequence homology of these oligonucleotides with DNA capture sites other than CAT01 (SEQ ID No. 1) and CAT02 (SEQ ID No. 2), respectively. In the case of CAT23B-CAT29B (SEQ ID Nos. 12-18), however, significant sequence homology results in a more complicated binding pattern.
- 30 Example 2. Detecting Oligonucleotides Directly Immobilized to Nitrocellulose
A. Immobilization of Oligonucleotides to Nitrocellulose
Synthetic oligonucleotides were haptenated using conventional methodologies and the resulting sequences are shown below in Table 2.1. The oligonucleotides were then individually diluted (1:1) in 20X SSC buffer (3 M
35 sodium chloride, 342 mM sodium citrate, pH 7.0) to yield three 150 µM solutions of each oligonucleotide.

-17-

TABLE 2.1

SEQ ID No.	5'	Sequence	3'
19		GAAATTGGCTCTTTAGCTTGTGTTTC-biotin	
20		AAACATGGAACATCCTTGTGGGGAC-biotin	
21		GACTTTCGATGTTGAGATTACTTTCCC-biotin	

Approximately 0.3 µl aliquots of each oligonucleotide solution were

- 5 dotted to the approximate middle of individual 0.4 cm x 5 cm strips of 0.45 µm
and 0.5 µm nitrocellulose available from Schleicher & Schuell; Keene, NH.
After the oligonucleotides were applied to the nitrocellulose, the nitrocellulose
strips were baked in an oven at 80°C for 20 minutes.

10 B. Detection of Immobilized Oligonucleotides

A rabbit anti-biotin selenium colloid conjugate was prepared as in Example 1 except the colloid was diluted 1:1 in distilled water (to yield an OD of 15 at a maximum wavelength of 546 nm) before it was added to the polyclonal antibody. The resulting conjugate was diluted 1:3 in 3% casein dissolved in tris® buffered saline (TBS - 100 mM tris, 150 mM NaCl, pH 7.8). 30 µl of the diluted conjugate was applied to one end of each of the nitrocellulose strips which had the oligonucleotides immobilized thereon. The conjugate was allowed to migrate along the length of the nitrocellulose strips (approximately 2 minutes) before an observation was made at which point a faint red dot was developing. After approximately 5 minutes, a red dot was observed on all of the nitrocellulose strips in the area where the oligonucleotides had been immobilized.

Example 3. Oligonucleotide Capture Using Oligonucleotides Directly

Immobilized to Nitrocellulose

25 A. Immobilization of Oligonucleotides

In this example, oligonucleotides were immobilized to nitrocellulose and employed to capture complementary single stranded DNA sequences or double stranded DNA sequences (obtained from Genosys, Woodlands, TX; and Synthecell, Columbia, MD). One strand of the double stranded DNA comprised a sequence complementary to the immobilized oligonucleotides. The oligonucleotides which were immobilized to the nitrocellulose strips can be found in Table 3.1.

-18-

TABLE 3.1

SEQ ID No.	5'	Sequence	3'
1		TATCATCTTGGTGT-NH ₂	
22		ACACCAAAAGATGATA	

The oligonucleotides were immobilized to nitrocellulose by dotting 150 μM solutions of the oligonucleotides onto approximately 0.4 cm x 5 cm strips of 5 μm nitrocellulose (Schleicher & Schuell). The immobilization procedure was the same as the procedure set forth above in Example 2 except that after the oligonucleotide solutions were applied to the nitrocellulose strips, the strips were baked at 75°C.

10

B. Hybridization of Immobilized Oligonucleotides and Their Cognates

Initially, the cognate oligonucleotides and the double stranded sequences comprising a complementary oligonucleotide (both of which will hereinafter be referred to as running oligonucleotides) were at a concentration of between 100 μM and 500 μM. The running oligonucleotides were diluted in 1% casein dissolved in 10 mM tris, 15 mM NaCl, pH 7.4. The sequences of the running oligonucleotides can be found in table 3.2.

TABLE 3.2

SEQ ID No.	Sequence
23	5' - ACACCAAAAGATGATA-fluorescein - 3'
24	5' - TATCATCTTGGTGT-fluorescein - 3'
25	5'-TATCATCTTGGTGT-fluorescein-3' 3'-ATAGTAGAACACACA-5'
26	5'-biotin-ACACCAAAAGATGATA-3'

20

Hybridization was achieved by applying 30 μl of the running oligonucleotides to one end of the nitrocellulose strips and allowing the oligonucleotides to migrate past the region containing the immobilized oligonucleotides (approximately 5 minutes). Detecting hybridization of the running oligonucleotides to the immobilized oligonucleotides was accomplished in one of two ways. One method included placing the strips under U.V. light and observing the situs of the immobilized oligonucleotides for a fluorescent

-19-

signal. The other method included contacting the ends of the nitrocellulose strips with a selenium colloid conjugate mixed with the running oligonucleotides, allowing the conjugate to migrate past the situs of the immobilized oligonucleotides, and observing the situs of the immobilized oligonucleotides for a visible signal. A signal in the region of the immobilized oligonucleotides indicated the hybridization of the running oligonucleotides and therefore the presence of the immobilized oligonucleotides in their original position. The selenium colloid conjugates comprised selenium colloid and an antibody specific for the label attached to the running oligonucleotide. Conjugates were prepared 5 as above in Example 2 using anti-fluorescein antibody and anti-biotin antibody.

Figure 4 is a photograph of nitrocellulose strips under a U.V. lamp after SEQ ID No. 23 migrated past the situs of immobilized SEQ ID No. 1. Figure 4(a) illustrates the fluorescent signal generated at the situs of the immobilized oligonucleotides when a 100 nM concentration of SEQ ID No. 23 was employed 10 as the running oligonucleotide. Figure 4(b) illustrates the fluorescent signal generated at the situs of the immobilized oligonucleotides when a 1 μ M concentration of SEQ ID No. 23 was employed as the running oligonucleotide. Figure 4(c) illustrates the fluorescent signal generated at the situs of the immobilized oligonucleotides when a 1.6 μ M concentration of SEQ ID No. 23 15 was employed as the running oligonucleotide. Figure 4(d) illustrates the fluorescent signal generated at the situs of the immobilized oligonucleotides when a 3.3 μ M concentration of SEQ ID No. 23 was employed as the running oligonucleotide. As illustrated by the signals observed in Figures 4(a)-(d), the running oligonucleotides hybridized to the immobilized oligonucleotides in the 20 region where they were originally applied.

Figure 5 illustrates the results obtained from another one step format where SEQ ID No. 22 was immobilized to nitrocellulose and SEQ ID No. 24 (single stranded DNA), at a 20 μ M concentration, and SEQ ID No. 25 (double stranded DNA), at a 20 μ M concentration, were used as the running 25 oligonucleotides at a 1:30 dilution in the running buffer (1% casein dissolved in 10 mM tris, 15 mM NaCl, pH 7.4). A selenium colloid anti-fluorescein conjugate (as prepared in Example 2) was employed to visually detect hybridization. Figure 5(a) shows the results after SEQ ID No. 24 and the selenium colloid conjugate had migrated past the situs where SEQ ID No. 22 had been immobilized. Similarly, Figure 5(b) shows the results after SEQ ID No. 30 35 25 and the selenium colloid conjugate had migrated past the situs where SEQ ID

-20-

No. 22 had been immobilized. As illustrated by Figures 5(a) and 5(b), hybridization was detected for the single stranded DNA (SEQ ID No. 22) but no hybridization was detected for the double stranded DNA (SEQ ID No. 25).

Figure 6 illustrates the results obtained when SEQ ID No. 1 was immobilized to three nitrocellulose strips and various concentrations of SEQ ID No. 26 were employed as a running oligonucleotide. Figure 6(a) illustrates the results obtained when the running oligonucleotide was applied to the end of a nitrocellulose strip at a 100 nM concentration. Figure 6(b) illustrates the results obtained when the running oligonucleotide was applied to the end of a nitrocellulose strip at a 1 nM concentration. Figure 6(c) illustrates the results obtained when the running oligonucleotide was applied to the end of a nitrocellulose strip at a 0.1 nM concentration. Hybridization of the running oligonucleotide to the immobilized oligonucleotide was detected visually, as above, using a selenium colloid anti-biotin conjugate (as prepared in Example 2). As shown by Figures 6(a)-6(c), hybridization occurred on all three strips as indicated by the signals observed in the regions where SEQ ID No. 1 was initially immobilized.

Example 4. Oligonucleotide Capture Using DNA & PNA Directly Immobilized to Glass

A. Directly Immobilizing Oligonucleotides and PNAs to Glass

Oligonucleotides were immobilized to 22 mm x 22 mm glass cover slips from Corning and employed to capture a cognate oligonucleotide. SEQ ID No. 27 was the DNA oligonucleotide immobilized to the glass cover slip and SEQ ID No. 28 was the PNA oligonucleotide immobilized to the glass cover slip. SEQ ID No. 27 was synthesized using conventional automated techniques and is listed below in Table 4.1. SEQ ID No. 28 was purchased from Millipore (Bedford, MA) and is listed below in Table 4.2.

30

TABLE 4.1

SEQ ID No.	5'	Sequence	3'
27		carbazole-GAGGTTGGTGAGTGA	

-21-

TABLE 4.2

SEQ ID No.	C-terminus	Sequence	N-terminus
28		fluorescein-GAGGTTGGTGAGTGA-NH ₂	

- Two dilutions of the oligonucleotide sequences were prepared. The
- 5 PNA was diluted in phosphate buffered saline (PBS) to yield solutions containing PNA concentrations of 44 μM and 11 μM. The DNA was diluted in PBS to yield solutions containing oligonucleotide concentrations of 37 μM and 14 μM. 1 μl aliquots of each dilution were then dispensed onto glass cover slips. The PNA and DNA solutions were allowed to dry onto the cover slip at
- 10 room temperature before the cover slip was baked at 60°C for 20 minutes. After the cover slip was baked, it was cooled to room temperature and overcoated with a 0.05% solution of alkaline treated casein dissolved in HPLC water. The overcoating lasted for one minute and excess casein was rinsed from the cover slip with HPLC water. Residual liquid on the cover slip was dried with forced
- 15 air.

B. Production of an Optical Waveguide

- Using double stick tape, a second 22 mm x 22 mm glass cover slip was secured (slightly off center) to the cover slips which had been spotted with the
- 20 oligonucleotide sequences. The channel formed between the two cover slips was approximately 0.75 μm deep and held approximately 25 μl of a liquid reagent. Two waveguides were produced in this manner.

C. Hybridization and Detection

- 25 A DNA sequence (Genosys) which was complementary to SEQ ID No. 27 (DNA) and SEQ ID No. 28 (PNA) was employed as a sample oligonucleotide. The sample oligonucleotide is designated SEQ ID No. 29 and is listed below in Table 4.3.

TABLE 4.3

SEQ ID No.	5'	Sequence	3'
29		biotin-TCACTCACCAACCTC	

-22-

Separate dilutions of SEQ ID No. 29 were made in buffers containing different concentrations of sodium phosphate. SEQ ID No. 29 was diluted to 170 nM in 1.5 mM phosphate buffer and 150 mM phosphate buffer. An aliquot large enough to fill the waveguide's channel, of each dilution was then 5 dispensed into the two waveguides produced above. The waveguides were then incubated at room temperature for 5 minutes in a humidity chamber at 100% humidity. After the incubation, the liquid in the waveguide was displaced with an anti-biotin selenium colloid conjugate which was prepared as above in Example 1 then the conjugate was diluted 1:1 with 10% alkaline treated casein in 10 distilled water. Immediately upon displacement, the waveguide was inserted into a 150 watt light source as in Example 1.

Figure 7 illustrates the results obtained when the waveguide was inserted into the light source. Figure 7(a) is a legend showing the dotting pattern used for the immobilization of the various concentrations of SEQ ID No. 27 and SEQ 15 ID No. 28. Figure 7(b) shows the waveguide results for SEQ ID No. 29 when diluted in 1.5 mM phosphate buffer. As shown by Figure 7(b), the signal was the greatest in the area where 44 μ M PNA was dotted. Figure 7(c) shows the waveguide results for SEQ ID No. 29 when diluted in 150 mM phosphate buffer. As shown by Figure 7(c), the signal was again the greatest in the area dotted with the 44 μ M PNA but the binding affinity for the DNA spots was 20 greater than in the 1.5 mM dilutions.

The above examples describe several specific embodiments of the invention but the invention is not restricted to these specific examples. Rather, 25 the invention to be protected is defined by the appended claims.

-23-

SEQUENCE LISTING

(1) GENERAL INFORMATION:

5 (i) APPLICANT: Tsung-Hui K. Jou
Joanell V. Hoijer

10 (ii) TITLE OF INVENTION: METHODS OF IMMOBILIZING OLIGONUCLEOTIDES
TO SOLID SUPPORT MATERIALS AND METHODS
OF USING SUPPORT BOUND OLIGONUCLEOTIDES

15 (iii) NUMBER OF SEQUENCES: 29

15 (iv) CORRESPONDENCE ADDRESS:

(A) ADDRESSEE: Abbott Laboratories
(B) STREET: 100 Abbott Park Road
(C) CITY: Abbott Park
(D) STATE: Illinois
20 (E) COUNTRY: USA
(F) ZIP: 60064-3500

25 (v) COMPUTER READABLE FORM:

(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: Macintosh
(C) OPERATING SYSTEM: System 7.0.1
25 (D) SOFTWARE: MS Word

30 (vi) CURRENT APPLICATION DATA:

(A) APPLICATION NUMBER:
(B) FILING DATE:
(C) CLASSIFICATION:

35 (viii) ATTORNEY/AGENT INFORMATION:

(A) NAME: Paul D. Yasger
(B) REGISTRATION NUMBER: 37,477
(C) DOCKET NUMBER: 5636.US.01

40 (ix) TELECOMMUNICATION INFORMATION:

(A) TELEPHONE: 708/937-4884
(B) TELEFAX: 708/938-2623
(C) TELEX:

45 (2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

50 (ii) MOLECULE TYPE: synthetic DNA

(ix) FEATURE:
(A) NAME/KEY: 3' amine
(B) LOCATION: 15

55 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

TATCATCTTT GGTGT

15

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:

-24-

- (A) LENGTH: 15 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
- 5 (ii) MOLECULE TYPE: synthetic DNA
(ix) FEATURE:
(A) NAME/KEY: 3' amine
(B) LOCATION: 15
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:
- 10 AATATCATTC GTGTT 15
- (2) INFORMATION FOR SEQ ID NO:3:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
- 15 (ii) MOLECULE TYPE: synthetic DNA
(ix) FEATURE:
(A) NAME/KEY: 3' amine
(B) LOCATION: 15
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:
- 20 AGTGGAGGTC AACGA 15
- (2) INFORMATION FOR SEQ ID NO:4:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 base pairs
(B) TYPE: nucleic acid
30 (C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: synthetic DNA
(ix) FEATURE:
(A) NAME/KEY: 3' amine
(B) LOCATION: 15
35 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:
- AGTGGAGATC AACGA 15
- (2) INFORMATION FOR SEQ ID NO:5:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 base pairs
(B) TYPE: nucleic acid
45 (C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: synthetic DNA
(ix) FEATURE:
(A) NAME/KEY: 3' amine
(B) LOCATION: 15
50 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:
- AGGTCAACGA GCAAG 15
- (2) INFORMATION FOR SEQ ID NO:6:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 base pairs
(B) TYPE: nucleic acid
55 (C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: synthetic DNA

-25-

(ix) FEATURE:
 (A) NAME/KEY: 3' amine
 (B) LOCATION: 15
 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

5

AGGTCAATGA GCAAG

15

(2) INFORMATION FOR SEQ ID NO:7:
 (i) SEQUENCE CHARACTERISTICS:

10 (A) LENGTH: 15 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

15 (ii) MOLECULE TYPE: synthetic DNA
 (ix) FEATURE:

(A) NAME/KEY: 3' amine
 (B) LOCATION: 15
 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

20

TGGAGATCAA TGAGC

15

(2) INFORMATION FOR SEQ ID NO:8:
 (i) SEQUENCE CHARACTERISTICS:

25 (A) LENGTH: 15 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: synthetic DNA

30 (ix) FEATURE:
 (A) NAME/KEY: 3' amine
 (B) LOCATION: 15

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

35

TGGAGATCAA CGAGC

15

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:

40 (A) LENGTH: 15 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(iii) MOLECULE TYPE: synthetic DNA

45 (ix) FEATURE:
 (A) NAME/KEY: 3' amine
 (B) LOCATION: 15

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

50

TGGAGGTCAA TGAGC

15

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:

55 (A) LENGTH: 15 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: synthetic DNA

(ix) FEATURE:
 (A) NAME/KEY: 3' biotin
 (B) LOCATION: 15

-26-

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

ACACCAAAGA TGATA

15

5 (2) INFORMATION FOR SEQ ID NO:11:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 15 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: synthetic DNA
- (ix) FEATURE:
 - (A) NAME/KEY: 3' biotin
 - (B) LOCATION: 15

15 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

AACACCAATG ATATT

15

20 (2) INFORMATION FOR SEQ ID NO:12:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 15 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: synthetic DNA
- (ix) FEATURE:
 - (A) NAME/KEY: 3' biotin
 - (B) LOCATION: 15
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

30 TCGTTGACCT CCACT

15

35 (2) INFORMATION FOR SEQ ID NO:13:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 15 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: synthetic DNA
- (ix) FEATURE:
 - (A) NAME/KEY: 3' biotin
 - (B) LOCATION: 15
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

45 TCGTTGATCT CCACT

15

50 (2) INFORMATION FOR SEQ ID NO:14:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 15 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: synthetic DNA
- (ix) FEATURE:
 - (A) NAME/KEY: 3' biotin
 - (B) LOCATION: 15
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

CTTGCTCGTT GACCT

15

-27-

- (2) INFORMATION FOR SEQ ID NO:15:
(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 15 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear
 (ii) MOLECULE TYPE: synthetic DNA
 (ix) FEATURE:
 (A) NAME/KEY: 3' biotin
 (B) LOCATION: 15
 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

15 CTTGCTCATT GACCT

15

- (2) INFORMATION FOR SEQ ID NO:16:
(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 15 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear
 (ii) MOLECULE TYPE: synthetic DNA
 (ix) FEATURE:
 (A) NAME/KEY: 3' biotin
 (B) LOCATION: 15
 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

25 GCTCATTGAT CTCCA

15

- 30 (2) INFORMATION FOR SEQ ID NO:17:
(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 15 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear
 (ii) MOLECULE TYPE: synthetic DNA
 (ix) FEATURE:
 (A) NAME/KEY: 3' biotin
 (B) LOCATION: 15
 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

40 GCTCGTTGAT CTCCA

15

- 45 (2) INFORMATION FOR SEQ ID NO:18:
(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 15 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear
 (ii) MOLECULE TYPE: synthetic DNA
 (ix) FEATURE:
 (A) NAME/KEY: 3' biotin
 (B) LOCATION: 15
 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

55 GCTCATTGAC CTCCA

15

- (2) INFORMATION FOR SEQ ID NO:19:

-28-

- 5 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 26 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear
 (ii) MOLECULE TYPE: synthetic DNA
 (ix) FEATURE:
 (A) NAME/KEY: 3' biotin
 (B) LOCATION: 26
10 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

GAAATTGGCT CTTTAGCTTG TGTTC

26

- 15 (2) INFORMATION FOR SEQ ID NO:20:
 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 25 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear
 (ii) MOLECULE TYPE: synthetic DNA
 (ix) FEATURE:
 (A) NAME/KEY: 3' biotin
 (B) LOCATION: 25
20 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

25 AACACATGGAA CATCCTTGTG GGGAC

25

- 30 (2) INFORMATION FOR SEQ ID NO:21:
 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 27 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear
 (ii) MOLECULE TYPE: synthetic DNA
 (ix) FEATURE:
 (A) NAME/KEY: 3' biotin
 (B) LOCATION: 27
35 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

40 GACTTTCGAT GTTGAGAGTTA CTTTCCC

27

- 45 (2) INFORMATION FOR SEQ ID NO:22:
 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 15 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear
 (ii) MOLECULE TYPE: synthetic DNA
 (ix) FEATURE:
 (A) NAME/KEY:
 (B) LOCATION:
50 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

55 ACACCAAAGA TGATA

15

- (2) INFORMATION FOR SEQ ID NO:23:
 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 15 base pairs
 (B) TYPE: nucleic acid

-29-

(C) STRANDEDNESS: single
 (D) TOPOLOGY: linear
 (ii) MOLECULE TYPE: synthetic DNA
 (ix) FEATURE:
 (A) NAME/KEY: 3' fluorescein
 (B) LOCATION: 15
 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

ACACCAAAGA TGATA

10 15

(2) INFORMATION FOR SEQ ID NO:24:
 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 15 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear
 (ii) MOLECULE TYPE: synthetic DNA
 (ix) FEATURE:
 (A) NAME/KEY: 3' fluorescein
 (B) LOCATION: 15
 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

TATCATCTTT GGTGT

15

25 (2) INFORMATION FOR SEQ ID NO:25:
 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 15 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: double
 (D) TOPOLOGY: linear
 (ii) MOLECULE TYPE: synthetic DNA
 (ix) FEATURE:
 (A) NAME/KEY: 3' fluorescein
 (B) LOCATION: 15
 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

TATCATCTTT GGTGT

15

40 (2) INFORMATION FOR SEQ ID NO:26:
 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 15 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear
 (ii) MOLECULE TYPE: synthetic DNA
 (ix) FEATURE:
 (A) NAME/KEY: 5' biotin
 (B) LOCATION: 1
 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

ACACCAAAGA TGATA

15

50 (2) INFORMATION FOR SEQ ID NO:27:
 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 15 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear
 (ii) MOLECULE TYPE: DNA

-30-

(ix) FEATURE:
(A) NAME/KEY: 5' carbazole
(B) LOCATION: 1
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:
5 GAGGTTGGTC AGTGA 15
(2) INFORMATION FOR SEQ ID NO:28:
(i) SEQUENCE CHARACTERISTICS:
10 (A) LENGTH: 15 base pairs
(B) TYPE: peptide nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: other (PNA)
15 (ix) FEATURE:
(A) NAME/KEY: peptide bond backbone
(B) LOCATION: 1-15
(ix) FEATURE:
20 (A) NAME/KEY: C-terminus fluorescein
(B) LOCATION: 1
(ix) FEATURE:
(A) NAME/KEY: N-terminus amino
(B) LOCATION: 15
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:
25 GAGGTTGGTC AGTGA 15
(2) INFORMATION FOR SEQ ID NO:29:
(i) SEQUENCE CHARACTERISTICS:
30 (A) LENGTH: 15 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: DNA
35 (ix) FEATURE:
(A) NAME/KEY: 5' biotin
(B) LOCATION: 1
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:
40 TCACTCACCA ACCTC 15

-31-

We claim:

1. A method of non-covalently immobilizing an oligonucleotide to a support material comprising the steps of:
 - (a) contacting an oligonucleotide solution with a solid support material wherein said oligonucleotides comprise between 5 nucleotides and 30 nucleotides; and
 - (b) drying said solution upon said solid support material.
2. The method of claim 1 wherein said solution has an oligonucleotide concentration of between 1 μ M and about 1 mM
3. The method of claim 1 wherein said solution further comprises a pH between 6.5 and 8.0, between 75 mM and 2 M sodium chloride, and a buffering system.
4. The method of claim 3 wherein said buffering system comprises between 10 mM and 250 mM tris®, sodium citrate or sodium phosphate.
5. The method of claim 1 wherein after said drying, said method further comprises baking said solid support material at a temperature between 60°C and 95°C.
6. The method of claim 1 wherein said oligonucleotides further comprise an amine group at the a 5' end or a 3' end.
7. The method of claim 1 wherein said oligonucleotides are peptide nucleic acids.
8. The method of claim 1 wherein after step (b), the method further comprises:
 - (i) contacting said solid support material with a conjugate; and
 - (ii) detecting a measurable signal as an indication of the presence or amount of said oligonucleotides.
9. The method of claim 1 wherein after step (b), the method further comprises:
 - (i) contacting said solid support material with a conjugate; and
 - (ii) detecting a measurable signal as an indication of the presence or amount of said conjugate.
10. The method of claim 1 wherein after step (b) the method further comprises:

-32-

- (i) contacting said solid support with a test sample suspected of containing nucleic acid sequences which are complementary to said oligonucleotides to form hybridization complexes;
- (ii) contacting said complexes with a conjugate; and
- (iii) detecting a measureable signal as an indication of the presence or amount of said nucleic acid sequences.

1/7

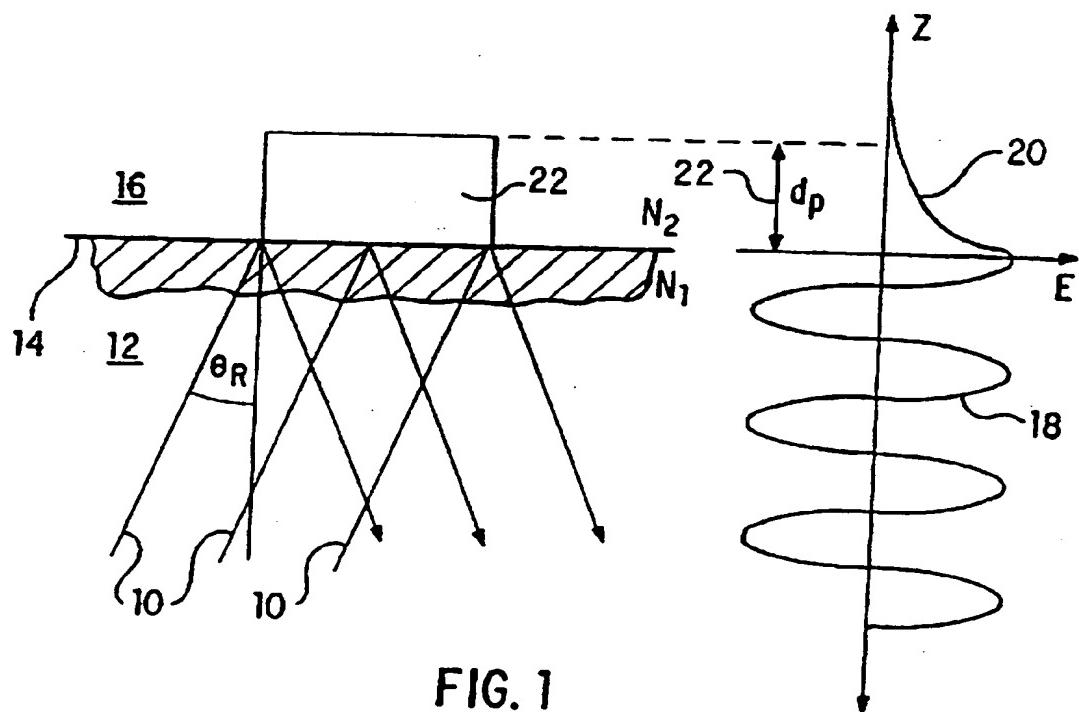


FIG. 1

2 / 7

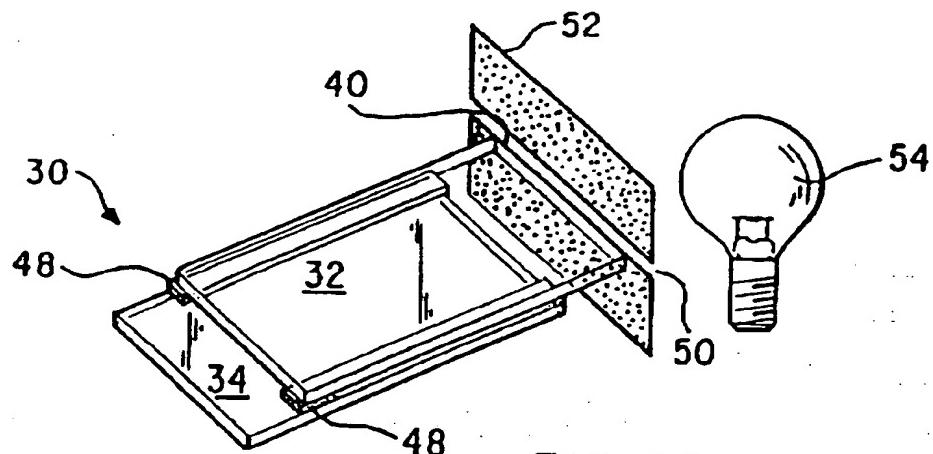


FIG. 2A

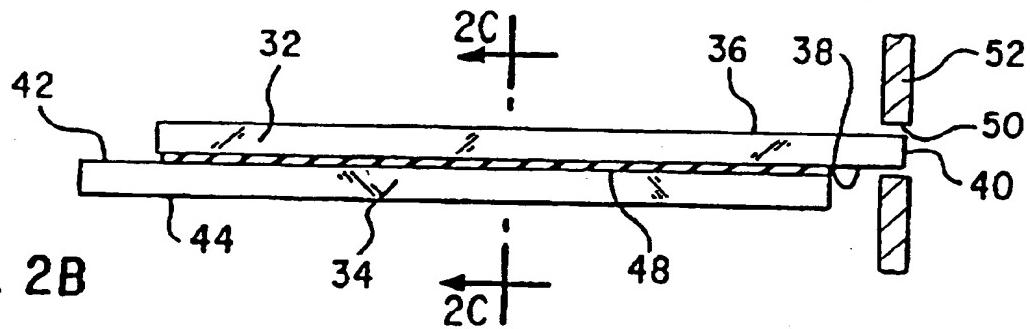


FIG. 2B

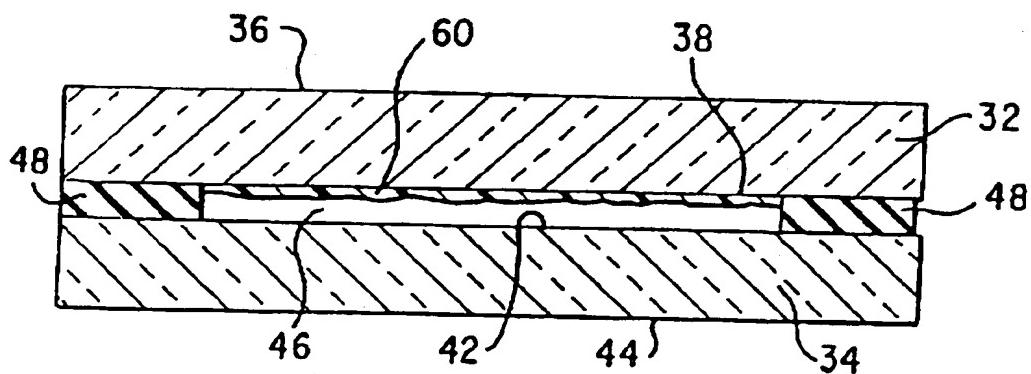


FIG. 2C

3/7



FIG. 3A

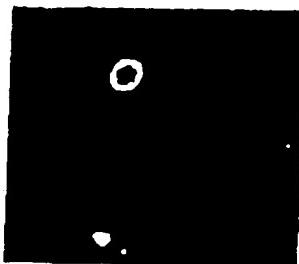


FIG. 3B

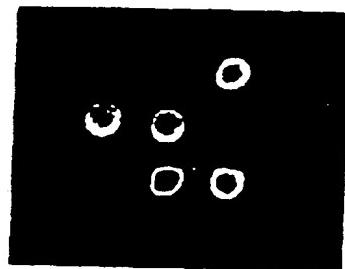


FIG. 3C

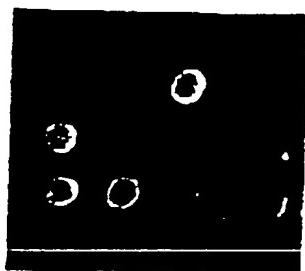


FIG. 3D

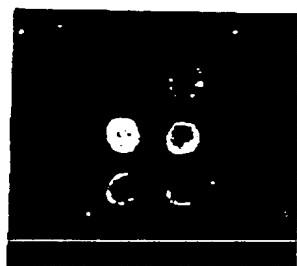


FIG. 3E

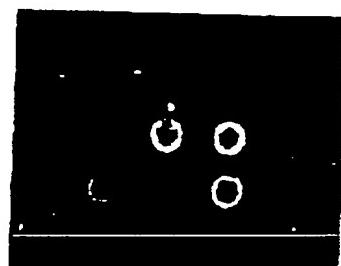


FIG. 3F



FIG. 3G



FIG. 3H

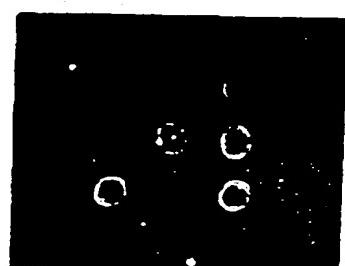


FIG. 3I

WO 96/19587

PCT/US95/16627

4/7

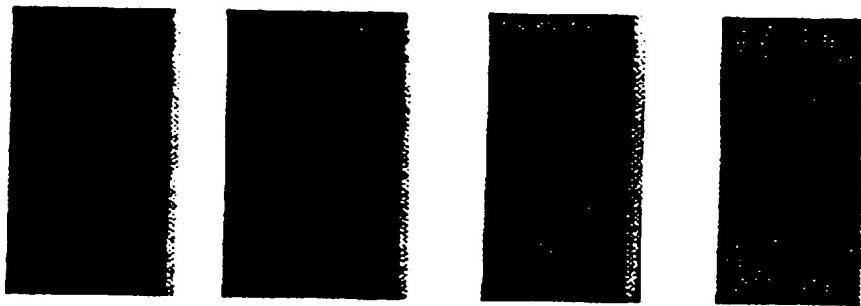


FIG.4A FIG.4B FIG.4C FIG.4D

WO 96/19587

PCT/US95/16627

5/7

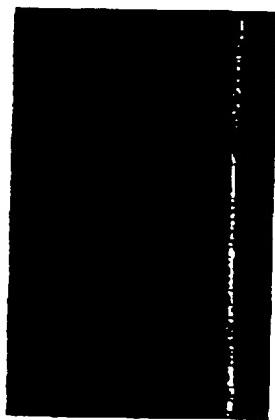


FIG. 5A

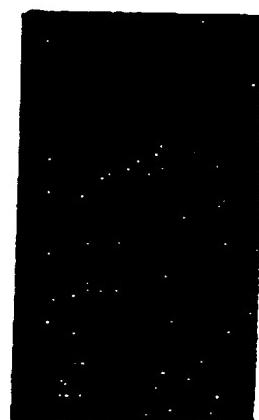


FIG. 5B

WO 96/19587

PCT/US95/16627

6/7

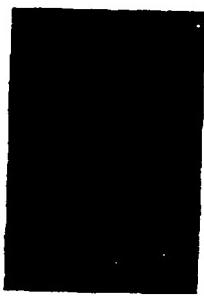


FIG.6A

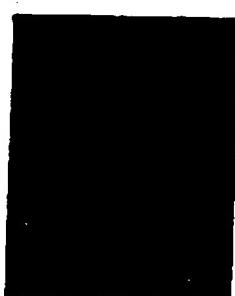


FIG.6B

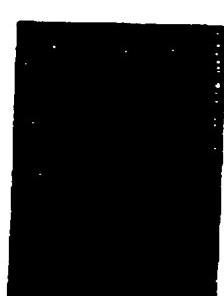


FIG.6C

7/7

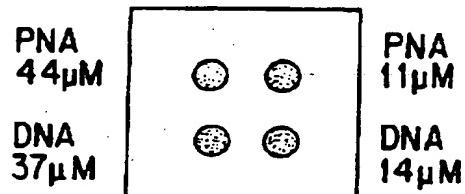


FIG.7A

FIG.7B



FIG.7C

